

Claims

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1 1. Recombinant enzymatically active
2 glucocerebrosidase produced by a eukaryotic cell.

1 2. The glucocerebrosidase of claim 1, produced
2 by an insect cell.

1 3. The glucocerebrosidase of claim 1, produced
2 by a mammalian cell.

1 4. The glucocerebrosidase of claim 3, produced
2 by a Chinese hamster ovary cell.

1 5. Recombinant enzymatically active
2 glucocerebrosidase comprising at least one exposed
3 mannose residue, said glucocerebrosidase being capable
4 of binding with a human mannose receptor protein.

1 6. The recombinant enzymatically active
2 glucocerebrosidase of claim 1 or 5, wherein said
3 glucocerebrosidase has an amino acid sequence with at
4 least 95% homology to an amino acid sequence of a
5 primate glucocerebrosidase.

1 7. The recombinant enzymatically active
2 glucocerebrosidase of claim 6, wherein said primate
3 glucocerebrosidase is human glucocerebrosidase.

1 8. The recombinant enzymatically active
2 glucocerebrosidase of claim 5, comprising at least two
3 exposed mannose residues.

1 9. The recombinant enzymatically active
2 glucocerebrosidase of claim 8, comprising a carbohydrate
3 moiety having between 3 and 9 exposed mannose residues.

1 10. The recombinant enzymatically active
2 glucocerebrosidase of claim 9, wherein said between 3
3 and 9 mannose residues are arranged in a Man₃ to
4 Man₉ structure.

1 11. The recombinant enzymatically active
2 glucocerebrosidase of claim 5, wherein said receptor
3 protein is a human mannose receptor protein occurring
4 naturally in a phagocytic cell.

1 12. The recombinant enzymatically active
2 glucocerebrosidase of claim 5, wherein said
3 glucocerebrosidase is produced within an insect cell.

1 13. The recombinant enzymatically active
2 glucocerebrosidase of claim 5, wherein said
3 glucocerebrosidase is produced within a mammalian cell.

1 14. A eukaryotic cell comprising nucleic acid
2 encoding enzymatically active glucocerebrosidase,
3 wherein said glucocerebrosidase is capable of
4 specifically binding with a human mannose receptor
5 protein.

1 15. The eukaryotic cell of claim 14, said cell
2 being an insect cell.

1 16. The eukaryotic cell of claim 14, said cell
2 being a mammalian cell.

1 17. The eukaryotic cell of claim 16, said
2 mammalian cell being a Chinese hamster ovary cell.

1 18. The eukaryotic cell of claim 14, wherein
2 said nucleic acid comprises DNA encoding human
3 glucocerebrosidase.

1 19. The eukaryotic cell of claim 14, wherein
2 said DNA lacks at least 50% of a naturally occurring
3 region between the promoter of said
4 glucocerebrosidase-encoding DNA and the ATG start site
5 of said glucocerebrosidase-encoding DNA.

1 20. The eukaryotic cell of claim 19, wherein
2 said cell is an insect cell.

1 21. The eukaryotic cell of claim 19, wherein
2 said cell is a mammalian cell.

1 22. The eukaryotic cell of claim 21, said
2 mammalian cell being a Chinese hamster ovary cell.

1 23. The insect cell of claim 15, wherein said
2 nucleic acid is provided by pVL941.GCRD21.

1 24. The insect cell of claim 15, wherein said
2 nucleic acid is provided by a vector comprising DNA
3 encoding an amino acid sequence having at least 95%
4 homology to an amino acid sequence of a naturally
5 occurring glucocerebrosidase.

1 25. The insect cell of claim 24, wherein said
2 nucleic acid is provided by pAc373.GCR2.2.

1 26. The insect cell of claim 24, wherein said
2 naturally occurring glucocerebrosidase occurs naturally
3 within a primate.

1 27. The insect cell of claim 26, wherein said
2 naturally occurring glucocerebrosidase occurs naturally
3 within a human.

1 28. The eukaryotic cell of claim 14, wherein
2 said glucocerebrosidase comprises at least two exposed
3 mannose residues.

1 29. The eukaryotic cell of claim 28, wherein
2 said glucocerebrosidase comprises a carbohydrate moiety
3 having between 3 and 9 mannose residues.

1 30. The eukaryotic cell of claim 29, wherein
2 said between 3 and 9 mannose residues are arranged in a
3 Man₃ to Man₉ structure.

1 31. An insect comprising a cell of claim 15.

1 32. A mammal comprising a cell of claim 16.

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33. A method for producing enzymatically
2 active glucocerebrosidase comprising the steps of:
3 introducing nucleic acid encoding
4 glucocerebrosidase into a eukaryotic cell;
5 causing said cell to express said
6 glucocerebrosidase; and
7 purifying said glucocerebrosidase.

1 34. The method of claim 33 wherein said
2 eukaryotic cell is an insect cell.

1 35. The method of claim 33 wherein said
2 eukaryotic cell is a mammalian cell.

1 36. The method of claim 35 wherein said
2 mammalian cell is a CHO cell.

1 37. The method of claim 33 wherein the step of
2 causing said cell to express said glucocerebrosidase
3 comprises culturing said cell in a culture medium in
4 vitro.

1 38. The method of claim 34 wherein the step of
2 causing said cell to express said glucocerebrosidase
3 comprises growing said cell in vivo within an insect.

1 39. The method of claim 35 wherein the step of
2 causing said cell to express said glucocerebrosidase
3 comprises growing said cell in vivo within a mammal.

1 40. The method of claim 37 wherein the step of
2 purifying said glucocerebrosidase comprises purifying
3 said glucocerebrosidase from said culture medium.

1 41. The method of claim 33 wherein the step of
2 purifying said glucocerebrosidase comprises disrupting
3 said cell to form a cellular extract and purifying said
4 glucocerebrosidase from said cellular extract.

1 42. The mammalian cell of claim ¹⁴16 wherein
2 said cell is transformed with any plasmid selected from
3 the group pGB20, pGB37, and pGB42.

1 43. The mammalian cell of claim 16 wherein
2 said cell is cotransformed with plasmid pGB34 and any
3 plasmid selected from the group pGB20, pGB37, and pGB42.

1 44. The method of claim 37 wherein the pH of
2 said culture medium is between about pH 6.5 and pH 7.2.

1 45. The method of claim 44 wherein the pH of
2 said culture medium is between about pH 6.6 and pH 6.8.

1 46. The method of claim 37 wherein said
2 culture medium contains O₂ in an amount below about
3 50% saturation and sufficient to maintain the cells.

1 47. The method of claim 37 wherein said
2 culture medium contains O₂ in an amount between about
3 20% saturation and about 30% saturation.